AMENDMENTS TO THE CLAIMS

Applicants have canceled claims 1-16 and added new claims 37-58. In addition, claims 17-36 have been withdrawn as being drawn to non-elected species.

1-16. (canceled)

17. (withdrawn) The GLP-1 (7-36) polypeptide and/or GLP-1 analog produced according to the method of claim 1.

18. (withdrawn) The GLP-1 (7-36) polypeptide according to claim 17, having an amino acid sequence of which is shown in Formula I:

Formula I His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-OH.

19. (withdrawn) A method of producing an expression vector comprising multiple tandem copies of a gene encoding a desired polypeptide comprising the steps of:

(a) constructing a vector comprising the gene and four individual restriction enzyme sites A-D in a relative order A-C-gene-B-D, wherein restriction enzyme sites C and B are capable of forming a hybrid site lacking restriction enzyme sites C and B;

(b) digesting an aliquot of the vector comprising the gene with endonucleases C and D and isolating a resulting double digested gene fragment;

(c) digesting a second aliquot of the vector with endonucleases B and D and isolating a resulting double digested vector including the gene;

(d) ligating the double digested gene fragment and the double digested vector comprising the gene to form a vector comprising N tandem copies of the gene linked by the hybrid site lacking restriction enzyme sites C and B; and

(e) repeating steps b-d, wherein each repeating series of steps begins with the vector product of step d such that the N tandem copies of the gene double with each series.

20. (withdrawn) The method of claim 19, wherein the gene encodes one or more additional N-terminal amino acids selected from the group consisting of: Met; Arg; Met-Arg; Met-Met-Arg; Asp-Asp-Asp-Asp-Lys; and combinations thereof.

21. (withdrawn) The method of claim 19, wherein the polypeptide is insulinotropic.

22. (withdrawn) The method of claim 21, wherein the insulinotropic polypeptide selected from the group consisting of: GLP-1(7-36); GLP-1 analogs; and exendin-4 analogs.

23. (withdrawn) The method of claim 22, wherein N is an integer from 2 to 32.

24. (withdrawn) The method according to claim 23, wherein N is an integer from 8 to 32.

25. (withdrawn) The method of claim 19, wherein restriction endonucleases sites C and B capable of forming a hybrid site are BglII and BamHI.

26. (withdrawn) The method of claim 19, wherein restriction endonucleases sites C and B capable of forming a hybrid site are SalI and XhoII.

27. (withdrawn) A method of producing an insulinotropic polypeptide comprising:

(a) expressing into a host cell a fusion protein comprising 2-32 tandem copies of the insulinotropic polypeptide, wherein each copy comprises a cleavable N-terminal Arg or cleavable spacer;

(b) isolating the fusion protein from the host cells;

(c) cleaving the fusion protein at the cleavable N-terminal Arg or cleavable spacer; and

(d) separating and purifying the insulinotropic polypeptide.

28. (withdrawn) The method of claim 27, wherein the fusion protein is cleaved by treatment with a compound selected from the group consisting of: cyanogen bromide, alkaline proteases, enterokinases, endopeptidases, and combinations thereof.

29. (withdrawn) The method of claim 28, wherein the alkaline protease is trypsin and internal lysine groups are acetylated prior to trypsin treatment.

30. (withdrawn) The method of claim 29, wherein the internal lysine groups are acetylated by treatment with an anhydride followed by deprotection after trypsin treatment.

31. (withdrawn) The method of claim 30, wherein the anhydride is selected from the group consisting of: acetic anhydride; maleic anhydride; citraconic anhydride, and 3,4,5,6-tetrahydrophthalic anhydride.

32. (withdrawn) The method of claim 27, wherein the insulinotropic polypeptide is selected from the group consisting of: GLP-1(7-36) (SEQ ID NO:1),

GLP-1(7-36)-NH_2 (SEQ ID NO:2), Gly^8-GLP-1(7-36) (SEQ ID NO:4), Val^8-GLP-1(7-36) (SEQ ID NO:5), Asp^{11}-GLP-1(7-36) (SEQ ID NO:6), Ala^{16}-GLP-1(7-36) (SEQ ID NO:7), Glu^{22}-GLP-1(7-36) (SEQ ID NO:8), His^{23}-GLP-1(7-36) (SEQ ID NO:9), Glu^{24}-GLP-1(7-36) (SEQ ID NO:10), Trp^{26}-GLP-1(7-36) (SEQ ID NO:11), Ala^{27}-GLP-1(7-36) (SEQ ID NO:12), Glu^{30}-GLP-1(7-36) (SEQ ID NO:13, Asp^{33}-GLP-1(7-36) (SEQ ID NO:14), Glu^{34}-GLP-1(7-36) (SEQ ID NO:15), Thr^{35}-GLP-1(7-36) (SEQ ID NO:16), Gly^8-Glu^{24}-GL- P-1(7-36) (SEQ ID NO:17), Leu^8-Ala^{33}-GLP-1(7-36) (SEQ ID NO:18), and exendin-4 analogs.

33. (withdrawn) The method of claim 32, wherein the insulinotropic polypeptide is GLP-1 (7-36) (SEQ ID NO 1) and the cleavage spacer is an N-terminal Arg.

34. (withdrawn) The method of claim 32, where the insulinotropic polypeptide is GLP-1 (7-36) (SEQ ID NO 1) and each GLP-1 copy is preceded by an N-terminal Met-Arg.

35. (withdrawn) The method of claim 34, wherein the isolated fusion protein is treated with cyanogen bromide followed by cleavage with clostripain protease.

36. (withdrawn) The method of claim 27, wherein the fusion protein has a coding sequence selected from the group consisting of: SEQ ID NO:29 and SEQ ID NO:30.

37. (new) A method of producing GLP-1 polypeptide comprising:

(a) providing a first nucleic acid,

wherein the first nucleic acid comprises a first gene which encodes one or multiple copies of the GLP-1(7-36) polypeptide or GLP-1 analog, a first and second restriction endonuclease sites located at one end of the first gene and a third and fourth restriction endonuclease sites located at the other end of the first gene,

wherein said second and third restriction endonuclease sites are located between said first and fourth restriction endonuclease sites,

wherein said second and third restriction endonuclease sites could form a hybrid site; and

wherein said first, second, third and fourth restriction endonuclease sites are recognized by a first, second, third and fourth restriction endonuclease, respectively;

(b) linearizing a vector with said first and fourth restriction endonucleases;

(c) ligating said vector resulting from step (b) with said first nucleic acid cleaved with said first and fourth restriction endonucleases;

(d) linearizing the vector resulting from step (c) with said third and fourth restriction endonucleases;

(e) providing a second nucleic acid,

wherein the second nucleic acid comprises a second gene which encodes one or multiple copies of the GLP-1(7-36) polypeptide or GLP-1 analog, said second restriction endonuclease site located at one end of the second gene and said third and fourth restriction endonuclease sites at the other end of the

second gene, wherein said third restriction endonuclease site is located between said second and fourth restriction endonuclease sites;

(f) ligating said vector resulting from step (d) with said second nucleic acid cleaved with said second and fourth restriction endonucleases;

(g) transforming said vector resulting from step (f) into a host cell; and

(h) expressing in the host cell a fusion protein comprising multiple copies of linked GLP-1(7-36) polypeptide or GLP-1 analog.

38. (new) The method of claim 37 further comprising the following steps after step (h):

(i) cleaving the fusion protein from step (h); and

(j) separating and purifying the GLP-1(7-36) polypeptide or GLP-1 analog.

39. (new) The method of claim 37 wherein steps (d) – (f) are repeated one or multiple times.

40. (new) The method of claim 37 wherein said first gene in step (a) encodes one copy of GLP-1(7-36) polypeptide or GLP-1 analog.

41. (new) The method of claim 37 wherein said first gene in step (a) encodes multiple copy of GLP-1(7-36) polypeptide or GLP-1 analog.

42. (new) The method of claim 37 wherein said second gene in step (e) encodes one copy of GLP-1(7-36) polypeptide or GLP-1 analog.

43. (new) The method of claim 37 wherein said second gene in step (e) encodes multiple copy of GLP-1(7-36) polypeptide or GLP-1 analog.

44. (new) The method of claim 37 wherein said second restriction endonuclease is BglII and said third restriction endonuclease is BamHI.

45. (new) The method of claim 37 wherein said second restriction endonuclease is BamH I and said third restriction endonuclease is BglII.

46. (new) The method of claim 37 wherein said second restriction endonuclease is Sal I and said third restriction endonuclease is XhoI.

47. (new) The method of claim 37 wherein said second restriction endonuclease is XhoI and said third restriction endonuclease is Sal I.

48. (new) The method of claim 37 wherein said host cell resulting from step (g) is the one contained in CGMCC Deposit No.0599.

49. (new) The method of claim 38 wherein said cleaving in step (i) is done with clostrispan or trypsin.

50. (new) A method of producing GLP-1 polypeptide comprising:

(a) providing a first nucleic acid,

wherein the first nucleic acid comprises a first gene which encodes one or multiple copies of the GLP-1(7-36) polypeptide or GLP-1 analog, a first restriction endonuclease site at one end and a second and third restriction endonuclease sites at the other end,

wherein said second restriction endonuclease site is located between said first and third restriction endonuclease sites on said first gene fragment,

wherein said first and second restriction endonuclease sites could form a hybrid site; and

wherein said first, second and third restriction endonuclease sites are recognized by a first, second and third restriction endonuclease, respectively;

(b) linearizing a vector with said first and third restriction endonucleases;

(c) ligating said vector resulting from step (b) with said first nucleic acid cleaved with said first and third restriction endonucleases;

(d) linearizing the vector resulting from step (c) with said second and third restriction endonucleases;

(e) providing a second nucleic acid,

wherein the second nucleic acid comprises a second gene which encodes one or multiple copies of the GLP-1(7-36) polypeptide or GLP-1 analog, said first restriction endonuclease site at one end and said second and third restriction endonuclease sites at the other end, wherein said second restriction endonuclease site is located between said first and third restriction endonuclease sites on said second gene fragment;

(f) ligating said vector resulting from step (d) with said second nucleic acid cleaved with said first and third restriction endonucleases;

(g) transforming said vector resulting from step (f) into a host cell; and

(h) expressing in the host cell a fusion protein comprising multiple copies of linked GLP-1(7-36) polypeptide or GLP-1 analog.

51. (new) The method of claim 50 further comprising the following steps after step (h):

(i) cleaving the fusion protein from step (h); and

(j) separating and purifying the GLP-1(7-36) polypeptide or GLP-1 analog.

52. (new) The method of claim 50 wherein steps (c) and (d) are repeated one or multiple times.

53. (new) The method of claim 50 wherein said first gene in step (a) encodes one copy of GLP-1(7-36) polypeptide or GLP-1 analog.

54. (new) The method of claim 50 wherein said second gene in step (d) encodes one copy of GLP-1(7-36) polypeptide or GLP-1 analog.

55. (new) The method of claim 50 wherein said first restriction endonuclease site is BglII and said second restriction endonuclease site is BamH I.

56. (new) The method of claim 50 wherein said first restriction endonuclease site is BamH I and said second restriction endonuclease site is BglII.

57. (new) The method of claim 50 wherein said first restriction endonuclease site is Sal I and said second restriction endonuclease site is XhoI.

58. (new) The method of claim 50 wherein said first restriction endonuclease site is XhoI and said second restriction endonuclease site is SalI.